WHITE-OCELLI—AN EXAMPLE OF A "SLIGHT" MUTANT CHARACTER WITH NORMAL VIABILITY.

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Most of the non-lethal mutant characters of *Drosophila melanogaster* may be described as "slight" in the amount of visible change. This is fortunate, for, in general, the mutant genes that cause great changes in an organ, or that cause several distinct changes in different organs, produce races of poorer viability than the wild type of the species. The amount of disturbance to viability is roughly proportional to the extent of the somatic change. In nature these "extreme" mutant types would not be able to survive in competition with the wild type, and in our experimental cultures their presence leads to aberrant ratios that tend to obscure the simple genetic relations. It is possible to reduce the shortage of these relatively inviable mutant characters only by making the conditions of culture exceptionally favorable. Thus, the number of eggs per culture must be restricted by breeding from a single mother; and the amount of food per culture should be carefully regulated. Great progress has been made in improving the quality and methods of using culture media. While it is possible by such improved methods to make the relatively inviable mutants usable in many kinds of experiments, there remain other experiments that require so high a degree of exactness or the simultaneous use of so many mutants that even one poorly viable mutant is inadmissible. As the number of mutant types increases, we are able to drop the use of more and more of the poorly viable mutants and to replace them by new mutants of normal viability. As already implied, these mutants of normal viability are nearly all characterized by somatic changes of slight degree, or by effects, so far as observable, upon a single organ only. Many of these "slight" characters are perfectly definite in demarkation and offer as great precision of classification as do more extreme mutant characters. Another great advantage of slight mutant characters is that they

do not preclude the simultaneous use of other mutants. On the other hand, the use of such an extreme mutant character as white-eyes prevents the effective use in the same experiment of all other eye colors, and likewise the use of vestigial-wing hinders the use of all other wing and venation characters. The most valuable mutants are, then, those of slight but definite somatic change, free from disturbance to viability or masking effects on other mutant characters.

Origin and Description of the White-ocelli Mutant.

One of the best examples of a slight mutant character that fulfills these conditions is that of "white-ocelli," which was found very early in the breeding work with *Drosophila* (June 21, 1912). The ocelli of wild flies are three small simple eyes in a group on the dorsal posterior part of the head. In color they are of a dilute brownish-red, about that of a coffee infusion. Close examination shows that the color of the ocelli themselves is quite light, about that of weak tea, but that there is a crescent-shaped deposit of dense brownish-red pigment about the median side of the two posterior ocelli and against the posterior side of the anterior ocellus. The apparent color of the ocellus is largely due to this outside pigment seen through the transparent lens-like ocellus, and consequently the color changes in intensity with the angle at which the ocellus is viewed.

It was observed that the ocelli of white and also of vermilion-eyed flies were without color or pigment deposit. These ocellar changes are only other effects of the white and vermilion genes. That the color of the ocelli could vary independently of that of the eyes became apparent when it was found that about half of the flies of the stock of the mutant black-body color had white ocelli, while the remainder had the normal coffee-colored ocelli. Some of the white-ocelli flies were bred together and gave a pure-breeding stock of white-ocelli flies (June, 1912). About a year after this, it was found (July, 1913) that the stock of the third-chromosome recessive spineless was also pure for the white-ocellar color. The gene for white-ocelli was thought to be in the third chromosome, since, in crosses in which the spineless was used, the white-ocellar character generally reappeared in association with the spineless, though sometimes not.

The usefulness of the mutant white-ocelli was not appreciated for some years after its discovery. This neglect was due, in large part, to the fact that the regular examination of flies during this period was carried out by aid of a hand lens only, and the separation of the white from the normal ocelli was difficult because of the small size of the region affected. The later work has been done with a binocular microscope, with special attention to proper illumination and magnification; and under these conditions the separation is complete and entirely accurate, though still somewhat slow.

The Linkage Relations of White-ocelli.

An accurate localization of the gene for white-ocelli was made easy by the use of the two excellent dominant characters dichæte and hairless. The locus of dichæte was near the left end of the map of the third chromosome as then known, being about 13 units to the right of sepia, while the locus of hairless was somewhat to the right of the middle (about 42 units to the right of sepia and 21 to the left of rough). Spineless white-ocelli males were outcrossed to dichæte hairless females; and the F_1 dichæte hairless females were back-crossed by spineless white-ocelli males, with the results shown below:

	0.		1*.		2.		3.		1, 2,		1, 3.		2, 3.		
1918 Sept. 27	D H	ss wo	D ss wo	H	D wo	ss H	D H wo	ss	D ss H	wo	D ss	H wo	D	ss H wo	Total
Total............	660	528	88	80	116	87	50	53	5	4	2	8	0	1	1,682

* Crossovers in the first region, that between dichæte and spineless, are headed by "1," etc.

The results of this experiment showed that the locus of white-ocelli is to the right of that of hairless by about 6.8 units (a total of 114 crossovers involving region three). The locus of white-ocelli, as thus established, is in what had been the longest unoccupied region of the third chromosome. There had been no workable mutant in the entire distance of about 20 units from ebony (1.5 units to the right of hairless) to rough (21.2 units to

the right of hairless). The ebony rough distance was so great that in constructing a map a correction was required on account of double crossing over. The presence of white-ocelli between ebony and rough gave an opportunity to make a direct test of the amount of double crossing over and consequently of the amount of correction required. The results of the spineless white-ocelli × hairless rough back-cross are given below:

	0.		1.		2.		3.		1, 3.		2, 3.		
1919 Feb. 25	ss wo	H ro	ss H ro	wo	ss ro	H wo	ss wo ro	H	ss H	wo ro	ss	H wo ro	Total
Total.............	1,084	1,126	163	195	118	91	226	255	11	9	0	1	3,179

There was 6.6 per cent. of crossing over between hairless and white-ocelli, which agrees with the value 6.8 found in the previous experiment. Likewise, the white-ocelli rough value of 15.8 is in agreement with the expectation from the usual value of 22 for hairless rough. There was only one double crossover in the hairless white-ocelli rough section—a percentage relatively very low. Comparisons show that in the third chromosome (as in the second) the region near the end of the chromosome has a far lower 'coincidence' than has the mid-region. The amount of correction of the observed crossover value for the hairless ebony interval is thus .063 per cent., or somewhat less than one tenth of one unit. Other back-cross tests involving this region have produced a total of 45,971 flies, of which 19.6 per cent. were crossovers. This value is to be corrected to 19.7, which is the map-distance between ebony and rough.

The Viability of the White-ocelli Mutant.

As just seen, the linkage of white-ocelli was worked out through use of the more convenient spineless white-ocelli stock, while the black white-ocelli stock was discarded. The white-ocelli character persisted in the original black stock. No effort was made to eliminate it, nor, on the other hand, to aid in its survival. In May, 1919, a census of the flies of this black stock showed that approximately half were white-ocellars. That is,

the character had persisted in undiminished frequency from June, 1912, to May, 1919, a period that represented fully 175 generations of flies. During this period the black stock had been carried on in mass-cultures. Every two weeks a new culture was started by transferring, without examination or selection, enough flies to insure breeding. In such mass-cultures overcrowding is extreme, and, in spite of the great numbers of parents, not many more offspring succeed in hatching than hatch from successful pair-cultures. The competition grows keener with the age of the culture, since the number of larvæ is continually increasing from eggs laid each day, while the quantity of available food soon begins to diminish and its quality becomes progressively poorer. The mass-culture method of breeding thus exercises a strong and continuous selection against the perpetuation of the weaker or slower hatching individuals or types. In several instances mixed stocks have been started with equal numbers of different mutations, and this stock transferred without selection through several generations. Watch was kept, and in these cases there has been a progressive change in the composition of the stock, rapid at first, until the numbers of one type were quite small, and thereafter slower but in the same direction. Recessive characters of very low viability may persist for many generations as a small proportion of the population. Their existence is maintained by the inter-crossing of the heterozygotes, whereby the mutant gene escapes the adverse selection that the mutant character suffers. Certain of our mutations are so sensitive to larval overcrowding that the ratios in mass-cultures and in pair-cultures seem to belong to different systems of heredity. Thus, the character strap approaches 1 in 4 in pair-cultures, but may approximate 1 in 16 in sister mass-cultures.

The persistence of the white-ocelli character in undiminished proportion through 175 generations of forced competition means that the mutant is under no disadvantage. Such a mutant might easily survive in nature, and one slightly advantageous might ultimately supplant the original type.

THE MODIFICATION OF EOSIN EYE COLOR BY WHITE-OCELLI.

An examination of the various stocks of eye color mutations showed that there was a strong correlation between the eye color and the ocellar color. The ocelli of white-eyed flies are entirely colorless. The ocelli of vermilion-eyed flies show a slight trace only of color. Indeed, in the case of vermilion, the vermilion gene has a relatively greater effect upon ocellar color than upon eye color. The ocellar color of pink is so faint that pink can not be used in the same experiment with white-ocelli without some confusion in classification. The ocellar color of the dark eye 'sepia' is itself also darker. In the ten multiple allelomorphs of the white series, the ocellar color is proportional to the eye color. This direct effect of eye color genes on ocellar color suggested that the reverse relation might also hold—namely, that the white-ocelli gene might dilute the eye color. A careful examination of the eye color of white-ocelli flies did not show any certain effect. White-ocelli was crossed to vermilion, and the F_2 vermilion white-ocelli flies were not distinguishable from the simple vermilion flies. In the F_2 of the cross between white-ocelli and eosin, a definite modification of the eosin by the white-ocelli gene was observed. In the case of the males, the eye color of the double form was lighter in intensity and less yellow in tone than that of the eosin brothers. In the females, the change was in the same direction but was less marked in degree. Probably 95 per cent. of the diluted males were separable from the simple eosin, while only about 60 per cent. of the females were thus separable. Eosin is known to be especially subject to specific modification,[1] and the effects of the white-ocellar gene give a color intensity and tone and a sexual difference practically identical with those observed in the case of the modifier 'pinkish.' The gene for pinkish was, however, in the second chromosome, and there are other differences between the two cases.

[1] See *Jour. Exp. Zoöl.*, July, 1919.